

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- | | | |
|-------------------------------------|-------------------------------------|--|
| ☐ | ☒ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ | Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Data analysis

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Raw sequence data have been deposited in NCBI SRA BioProject: PRJNA656311. Quality-controlled and filtered VCF's and the full outputs for the GWAS and evolutionary genetics statistical analysis are available on Dryad <https://doi.org/10.5061/dryad.tht76hf50>

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Use the terms *sex* (biological attribute) and *gender* (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.

Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.

Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).

Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)

Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

The study involved collection of *Sceloporus undulatus* individuals from three populations: AL (n=381), TN (n=20), and AR (n=20) for whole genomic sequencing.

Research sample

Sceloporus undulatus from AL (n=381) represent a population exposed to the red imported fire ant. Animals from TN and AR represent populations unexposed to the red imported fire ant. All animals were sexually mature and of either sex.

Sampling strategy

Animals were collected as available, and the sample size was determined by budget limitations in whole genome sequencing.

Data collection

Genomic data was collected via genomic sequencing. Phenotypic data (limb length, sex, and body length) was collected by various researchers throughout 2006 and 2017.

Timing and spatial scale

Animals were collected on Summers of each year between 2006 and 2017.

Data exclusions

One animal was excluded from the analysis due to pre-established criteria of genomic data quality control (missing genotype rate > 10%).

Reproducibility

This sampling effort and analysis was the only attempt to detect signatures of adaptation in fence lizards to invasive fire ants.

Randomization

Randomization was not relevant to this study because it involved cross-population comparisons.

Blinding

Blinding for phenotypic data was not possible because phenotypic data were collected prior to the current experiment being designed. Further, all genomic data was processed identically across groups.

Did the study involve field work?

☒ Yes ☐ No

Field work, collection and transport

Field conditions	Animals were collected during summers, under sunny or overcast conditions, but not during rain
Location	Solon Dixon Forestry Education Center, 31°09'N 86°42'W; Edgar Evins State Park, 36°05'N 85°48'W; Lee County, AR 34°42'N 90°43'W
Access & import/export	Collection permits were granted by the Alabama Department of Conservation and Natural resources, the Arkansas Game & Fish Commission, and the Tennessee Department of Environment and Conservation, for each collection year. Alabama Department of Conservation and Natural resources: permit numbers 3568 (12/27/2005), 4059 (2/16/2007), 4454 (2/21/2008), 6521 (04/2009), 7564 (04/2010), 7762 (05/05/2011), 8271 (4/19/2012), 8680 (5/2014), 8812 (4/29/2015), 9311 (04/2016), 9585 (4/2017). Tennessee Department of Environment and Conservation: permit number 3547 (4/21/2011 and 4/10/2013). Arkansas Game and Fish Commission: permit number 022520081 (3/2006).
Disturbance	We accessed all habitats in a responsible manner and in compliance with local, national and international laws. There was no significant disturbance except for removal of study individuals from their habitat.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	The study did not involve laboratory animals.
Wild animals	All individuals (<i>Sceloporus undulatus</i>) were collected by slipknot lasso and transported in cloth bags. Age of individuals is unknown, but all were sexually mature (snout-to-vent length > 54mm). After tissue collection at any given year, all animals were released at the site of capture.
Reporting on sex	Findings apply to males and females, which were part of this study. Sex was identified by the presence of post-anal scales (males). Sex information is available in the open access data sets. Numbers are 211 females and 170 males for the Alabama population, 10 females and 10 males for the Tennessee population, and 11 females and 9 males for the Arkansas population.
Field-collected samples	Tissue samples were collected from the field and preserved in 70% ethanol until DNA extraction.
Ethics oversight	Animal collection adhered to Guidelines for the use of Animals in Research, the legal requirements of the U.S.A., the Institutional Guidelines of The Pennsylvania State University, and were approved by the Institutional Animal Care and Use Committee (IACUC).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.